SOME ASPECTS OF PHYSIOLOGICAL AGING IN THE ADULT WORKER HONEY BEE [1, 2]

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In reviews on the general physiology of aging, Lansing (1947, 1951) has emphasized the need for study at a cellular level of the problem of senescence, a phenomenon demonstrating a uniform pattern in a wide variety of organisms. This concept encompasses examination of anatomical and morphological, degenerative alterations, as well as biochemical changes such as hormonal and enzymic perturbations accompanying the involutionary process of growing old. Such studies must necessarily involve a long-term investigation of the various facets of the phenomenon of aging in animals or individuals whose complete medico-ecological history is a matter of record. To this end the worker honey bee (*Apis mellifera*) represents an animal which can be obtained in large numbers and which can be maintained during its life span under controlled physical environmental conditions with a minimum of care, while occupying relatively little space.

In an earlier study, Rockstein (1950) reported that the number of cells at two representative levels of the brain of the adult worker bee decreased steadily from the day of emergence to old age, whereas the activity in total brain homogenates of the enzyme cholinesterase rose during the first week to ten days following emergence and remained at this elevated level throughout the remainder of the life of the bee. This indicated the absence of a significant role by this enzyme system in the physiology of aging, from the standpoint of senescence.

Important in many aspects of intermediary metabolism, specifically in vital processes like nucleic acid and carbohydrate metabolism (see Moog, 1946 and Roche, 1950), the phosphomonoesterases suggested another enzyme system of sufficiently elevated importance which might prove related to the process of senescence. In pursuing this problem further, the writer has therefore studied changes in activity of acid and alkaline sodium β-glycerophosphatases in total body homogenates, as a function of age in the adult worker honey bee, *Apis mellifera* L., the results of which studies are presented herewith.

Methods

Frames of sealed worker brood were removed from a colony in the college apiary to an incubator maintained at 32.5° C. Adult bees in large numbers (2000 or more) were removed within 24 hours after emergence and were maintained

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thereafter in a large screen cage, under conditions of continuous artificial lightining and at a constant temperature of 26.5° C. and relative humidity of 50%, with adequate pollen, honey and water supplies for *ad libitum* feeding. At definite intervals of time, bees of known age were removed and homogenized in lots of twenty by the procedure described by Rockstein and Herron (1951), and the acid and alkaline phosphatase activity determined, respectively, as described by Rockstein and Levine (1951) and Rockstein and Inashima (1953). Determinations on very old bees were deferred until the point where the cage population began to show a rapid decline and where just enough live material was available.

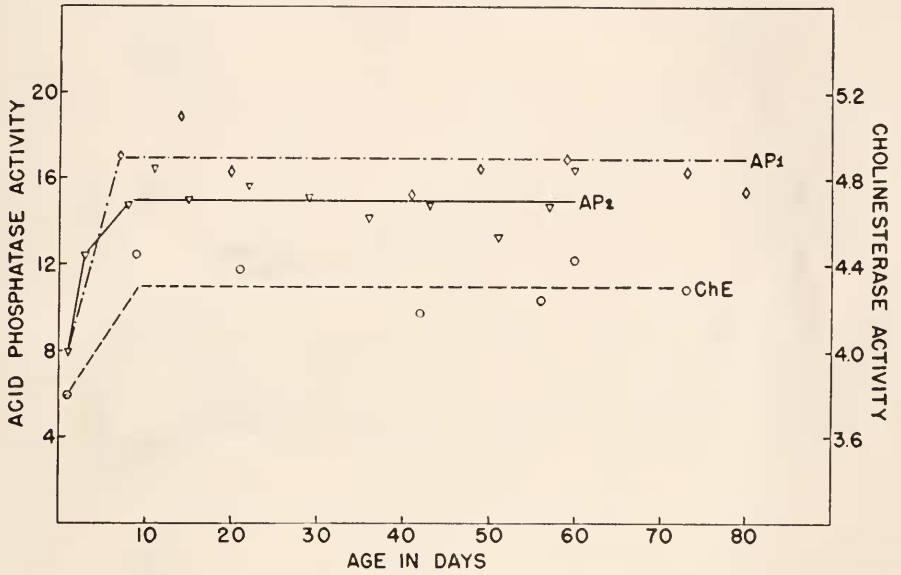

FIGURE 1. Acid phosphatase in whole body and cholinesterase in whole brain homogenates of the adult worker honey bee.

RESULTS

Figure 1 shows two curves labelled "AP_1" and "AP_2", denoting acid phosphatase studies of whole body homogenates for two populations of bees, as compared to data from the earlier report by the author (1950), averaged and plotted for cholinesterase activity in whole brain homogenates. Phosphatase activity is expressed as micrograms of phosphate as phosphorus, released in 1½ hours at 35° C. at pH 5.4, in a 0.2 ml. sample of deproteinized incubation mixture (see Rockstein and Levine, 1951, for details); cholinesterase activity is expressed as micromoles of acetylcholine bromide hydrolyzed per whole brain per hour at 30° C. During the first eight to ten days of adult life the total body acid enzyme shows a rise in activity representing an increase of about 90%; during the corresponding period the total brain cholinesterase shows a parallel rise in activity, but of only 14%. For both enzymes this elevated activity appears to remain undiminished from about ten days to very old age.

Figure 2 shows similar curves for the alkaline enzyme (only one series) and collated data for brain cell number, from the author's earlier report (1950), averaged and plotted for comparison. Alkaline enzyme units are the same as for the acid enzyme, representing activity under identical conditions except that the pH of incubation was maintained at 8.1. Here is seen a steady decrease in brain cell number to the extent of about a 35% decline in old bees from the original cell count in day-old bees; total body alkaline enzyme, however, shows a steady decline

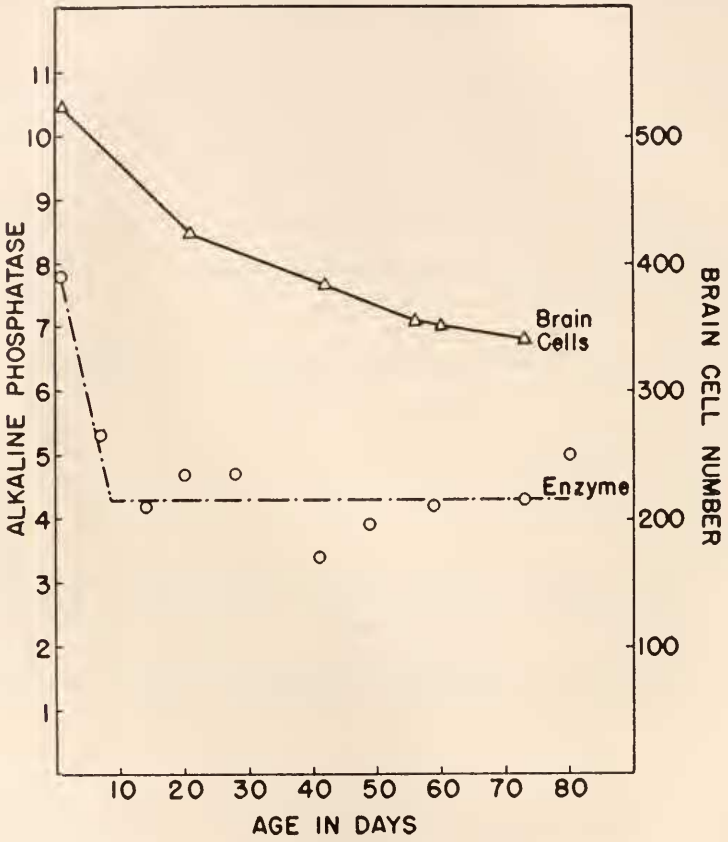

FIGURE 2. Alkaline phosphatase in whole body homogenates and brain cell number in the adult worker honey bee.

during the first week to ten days to a level 44% below that of day-old bees, following which no significant change occurs until old age.

Each point plotted for phosphatase activity in both figures represents the median value for at least five replicate determinations from each homogenate preparation.

DISCUSSION

If the total aging process is accepted to be an outward manifestation of inner biochemical alterations at the cellular level, the immediately controlling process or

processes may be more precisely defined in terms of modifications in the activity of identifiable enzyme systems catalyzing important metabolic processes. One agrees that correlation of data, whether it be statistically or graphically demonstrable, does not guarantee causality for phenomena under consideration, biological or otherwise. However, as in the case of cholinesterase, an enzyme important in the metabolism of acetylcholine (itself important in the mediation of the nerve impulse in certain parts of the nervous system of most animals), deductions can be made with some degree of certainty concerning their rationality.

From the earlier study by Rockstein (1950), no such positive relationship was indicated between senescence and brain cholinesterase in the honey bee, but rather some positive part played by that enzyme in post-embryonic maturation of the neuromotor mechanism.

Data in this report indicate no direct relationship between the alkaline and acid phosphomonoesterases and senescence; however, if the alkaline is indeed involved its effects must be quite indirect; *i.e.*, its decline at an early stage of adult life may be the forerunner of a gradual biochemical decline eliciting more directly the outward manifestations of senescence. This suggestion is in harmony with the concept of aging as a process initiated rather early in post-embryonic life of an individual and proceeding through several phases which include progressive maturation and senescence.

As has been mentioned elsewhere (Rockstein and Herron, 1951), Moog (1946) proposed that the presence of acid and alkaline phosphomonoesterases signifies a dual, matching dephosphorylating mechanism in the intermediary metabolism of glycogen. In the adult worker honey bee, data presented in this report point to a singular reciprocal relationship between the acid and alkaline sodium β-glycerophosphatases; *i.e.*, the acid enzyme shows a rise in activity during the first week to ten days following emergence while the alkaline enzyme shows a corresponding fall in activity during this same period of time. The fact is that this represents a post-larval period in this species, during which the power of flight is apparently being developed to its maximum. Wing beat frequency in *Drosophila* is reported by Sacktor (1950) as having been found by Chadwick and Williams (unpublished data) to be low immediately following emergence and to increase during the first few days of adult age to a level maintained throughout adult life; Sacktor himself found a marked increase in the activity of cytochrome oxidase, in total body homogenates of DDT-resistant and normal strains of the common house fly, the second day after adult emergence. The fact that brain cholinesterase in the adult honey bee also shows a strikingly parallel increase during the first week to ten days of adult life suggests that the interrelationship among the three enzyme systems, mentioned for the honey bee in this report, may be part of a well-integrated pattern of biochemical alterations concerned with completion of development of adult characteristics, particularly the neuromotor mechanism of strong-flying species. In this connection, evidence has been presented in an earlier discussion by Rockstein (1950), as well as in reports by Nachmansohn (1939), Sawyer (1943a, 1943b, 1944), Welsh and Hyde (1944) and Lindeman (1945), concerning the correlation between cholinesterase activity and the attainment of the ability to perform rapid movements in whole embryos, in developing immature young, and in different species of animals with varying degrees of motor ability.

Watanabe and Williams (1951) reported that the cytochrome oxidase activity in isolated sarcosomes (giant mitochondria) of the flight muscles of *Phormia regina* showed a pronounced drop (about 50%) during the first three to four days following adult emergence, at which reduced level of activity the enzyme in question remained constant thereafter. The variance between their data and those of Sacktor (1950) for total homogenates of the house fly indicates a need for further study of this enzyme in the house fly at the tissue level. Watanabe and Williams also reported a precipitous decline in catalase activity of sarcosomes during the similar three-four day period following emergence to about 20% of the original activity of a newly emerged adult fly, which decline was followed by a gradual dropping off to a low level of about 10% of the original activity at the end of the third week of adult life. The pattern of the latter findings suggests a possible basis for re-examination of the biochemical picture in the honey bee with regard to catalase, as well as the phosphatases, at the organ or tissue level, for possible further clarification of the maturation process during the post-emergence period in this species, and in other insects with a strong flight pattern.

Summary

1. Acid sodium β-glycerophosphatase in whole body homogenates of adult worker honey bees shows a rise in activity by about 90% from the first to the tenth day following emergence and remains unchanged thereafter at this elevated level until old age.

2. Alkaline phosphatase shows a steady decline in activity to about 44% below that of day-old bees, by about the tenth day following emergence, remaining unchanged at the lowered level until old age.

3. Although no direct relationship between these enzymes and the process of senescence is apparent, the correspondence between these data and earlier findings is discussed in terms of the post-emergence development of the neuromotor mechanisms in vigorous-flying holometabolous insects.

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